We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

4,800
Open access books available

122,000
International authors and editors

135M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

CLARIVATE ANALYTICS BOOK CITATION INDEX INDEXED

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Chapter

Fatty Acids: From Membrane Ingredients to Signaling Molecules

Michio Hashimoto and Shahdat Hossain

Abstract

Fatty acid constitutes the foundation cell membranes, provides metabolic energy, affects functions of membrane-bound enzymes/receptors, conducts signaling cascades, and helps in learning-related memory cognition in mammals, including humans. Structurally, the fatty acids are of two kinds: saturated and unsaturated; the latter are again of mono- and polyunsaturated types. From nutritional perspectives, they are of essential and nonessential types. Omega-6 linoleic acid (ω-6 LLA, C18:2) and ω-3 alpha linolenic acid (ω-3 αLLN, C18:3) and ω-6 arachidonic acid [(ω-6 AA, C20:4); it is conditional] are essential fatty acids (EFAs). In addition, mammalian brains cannot biosynthesize the ω-3 docosahexaenoic acid (ω-3 DHA, C22:6) in adequate amounts because of lack of necessary enzymes. Thus, DHA is essential for the growth and development of the brains. Deficiency of DHA produces visual- and learning-related memory impairments, and neurodegeneration in the aged brains and Alzheimer's disease brains. Finally, this chapter will highlight and broaden the awareness about the essentiality of different fatty acids with a special emphasis on DHA.

Keywords: docosahexaenoic acid, eicosapentaenoic acid, arachidonic acid, alpha-linolenic acid and linoleic acid, eicosanoids, docasonoids, brain cognition

1. Introduction

The concept of fatty acid was first introduced by the French chemist Michel Eugène Chevreul as *graisse acide* (acidic fat) [1]. Fatty acids are chemically defined as carboxylic acids with either saturated or unsaturated aliphatic chains and are derived after hydrolysis of fats or oils. A fatty acid has, therefore, an acid group at one end of its molecule and a methyl group at the other end [2, 3]. Fatty acids are essential structural components of the cell; they also play important roles in energy requirements and signaling cascades in the cell. Both plant and animal cells can synthesize fatty acids. Animal cells, however, cannot synthesize some of the fatty acids; they must take them from plant sources. These fatty acids are called essential fatty acids (EFAs) in the animal body. Some fatty acids are also synthesized by lower organisms such as phytoplanktons, which act as primary members of the food chain. On the basis of the location of the double bonds from the methyl terminal position of the unsaturated fatty acids (UFAs), they are named as ω-3 and ω-6 UFAs. Biologically, fatty acids are esterified with glycerol, phosphoglycerol, and cholesterol and are referred to as triacylglycerol, phospholipids, and cholesterol esters, respectively. Esterified fatty acids can constitute the structural components

or dietary fuels for cells and organisms; they can also form complex liposomal structures (including lipoproteins) for transporting lipid components from the hepatic tissues to extrahepatic tissues and vice versa.

1.1 Saturated versus unsaturated fatty acids

Fatty acids whose aliphatic carbon chains are fully saturated with hydrogen atoms or contain only C-C single bond and/or contain no C=C double bonds are simply referred to as saturated fatty acids (SFAs). Fatty acids containing C=C double bonds are referred to as unsaturated fatty acids (UFAs). UFAs are again classified as monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs): if they contain only one C=C double bond, they are MUFAs; if they contain more than one C=C double bond, they are then called PUFAs (see **Figure 1** for detail). Because of the presence of C-C single bonds or C=C double bonds, they have characteristic structural features and differences in physical as well as chemical properties and have significant roles in the constitution of cellular membranes.

1.2 Omega-3 (ω-3) versus omega-6 (ω-6) PUFAs

The Greek letter omega (ω) is used in the systemic nomenclature of the polyunsaturated fatty acids (PUFAs). The PUFAs that have a C=C double bond between the 6th and 7th carbon position counting from the terminal methyl end are called ω-6 and those with the double bond between the 3th and 4th carbon are called ω-3 PUFAs. The letter 'n' is also used to denote the position of the double bond. The locations of double bonds in the PUFAs confer huge differences in their physical, biochemical, and physiological properties. The essential fatty acid (EFA) linoleic acid (C18:2) is of ω-6 series, while the EFA α-linolenic acid is the member of ω-3 series. Some of the beneficial effects overlap between the ω-3 and ω-6, while many effects are antagonistic to each other. ω-6 PUFAs can be found in vegetable oils and seeds, whereas ω-3 PUFA is found more in fish/marine animals, walnuts, and canola oil.

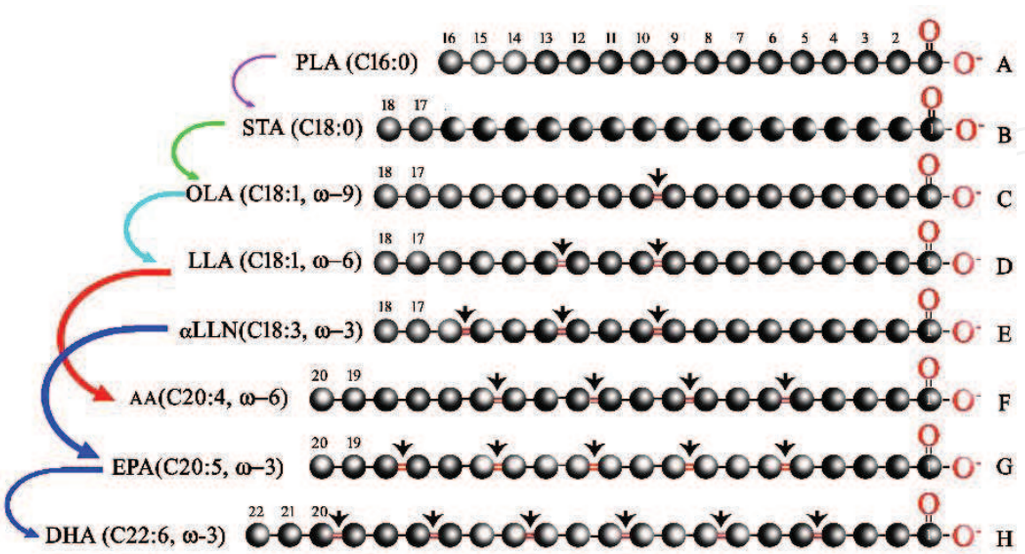

Figure 1.
The straight chain structural features of the most common fatty acids. PLA = palmitic acid, STA = stearic acid, OLA = oleic acid, LLA = linoleic acid, LLN = α-linolenic acid, AA = arachidonic acid, EPA = eicosapentaenoic acid, DHA = docosahexaenoic acid. Omega (ω) is used to denote the position of double bonds from the methyl end of the fatty acid. Colored curved arrows = biological conversion is possible from the precursor by the actions of elongase/desaturase enzymes. Black arrow = indicates the position(s) of double bond.

1.3 Essential versus nonessential fatty acids

The fatty acids, which mammals cannot synthesize in their body, are known as essential fatty acids (EFAs); they must be obtained by the mammals in a preformed condition, that is, from the exogenous dietary sources. EFAs were originally designated as vitamin F, until it was realized that they must be classified with fats [4]. Of all the 18-C UFAs, two unsaturated fatty acids are found to be essential fatty acids (EFAs): they are linoleic acid (**Figure 1D**) and α-linolenic acid (**Figure 1E**). Both of them can act as precursors of very long chain polyunsaturated fatty acids (LPUFAs), such as ω-6 linoleic acid acting as the precursor of arachidonic acid (C20:4, ω-6) and ω-3 α-linolenic acid acting as the precursor of eicosapentaenoic acid (EPA, C20:5, ω-3) and docosahexaenoic acid (DHA, C22:6, ω-3). The rest are nonessential. Some examples are (common names): stearic (C18:0), oleic (C18:1), palmitic (C16:0), myristic (C14:0), and lauric acid (C12:0). Being nonessential does not actually mean that they are not important. Our body does need them to function properly; it, however, can synthesize them without receiving them directly from food.

1.4 AA (C20:4, ω-6) versus DHA (C22:6, ω-3) or EPA (C20:5, ω-3)

AA is referred to as a conditionally essential fatty acid for animals [5–7], including humans, that experience persistent deficiencies of linoleic acid (LLA, C18:2, ω-6), or during prematurity and growth, or if there is a limited capacity to convert LLA to AA [5]. However, consumption of vegetable-based oils, with large amounts of LLA, and an adequate capacity to convert LLA to AA, can eliminate the need for exogenous supply of AA, excluding it thereby from the list of essential fatty acids.

1.5 EPA (C20:5, ω-3) and DHA (C22:6, ω-3)

Both EPA and DHA are the members of ω-3 PUFA family. Both can be biosynthesized from the precursor α-linolenic acid (C18:3, ω-3, LLN). However, they are believed to act differently in different organs. For example, the differential roles of EPA and DHA have been studied in lymphocytes [8], macrophages [9], vascular smooth muscle cells [10], and endothelial cells [11]. Their differential roles have also been seen in the brains. EPA constitutes a tiny part in the unsaturated fatty acid pool of the brain. DHA, however, constitutes >17% by weight of the total fatty acids in the brain of adult rats and >33% of the total fatty acids in the retina [12]. DHA is thus referred to as essential for the growth and development of the brains, and animals have to take it in preformed form. The brain has a limited capacity to convert αLNN to DHA because of the lack of synthesizing enzymes [13, 14]. DHA plays an important role in the learning-related memory of animals, including humans.

1.6 ω-7 and ω-9 Monounsaturated fatty acids (MUFAs)

Monounsaturated ω-7 and ω-9 fatty acids are also considered to be nonessential, as majority of them are obtained from dietary sources (**Figure 2**). They can also be biosynthesized in the body. The most common ω-7s are palmitoleic acid (PA) and *cis* and *trans*-vaccenic acid (VA) (11-cis-octadecenoic acid). The most common ω-9s include oleic acid (OA), erucic acid (EA) and mead acid (it is a triunsaturated fatty acid). Since the human body can create ω-9 unsaturated fatty acids, there is no need to include them in diet. Full-fat grass-fed dairy, wild-caught salmon, olives, sprouted nuts, etc. are the sources of ω-7 and ω-9 unsaturated fatty acids.

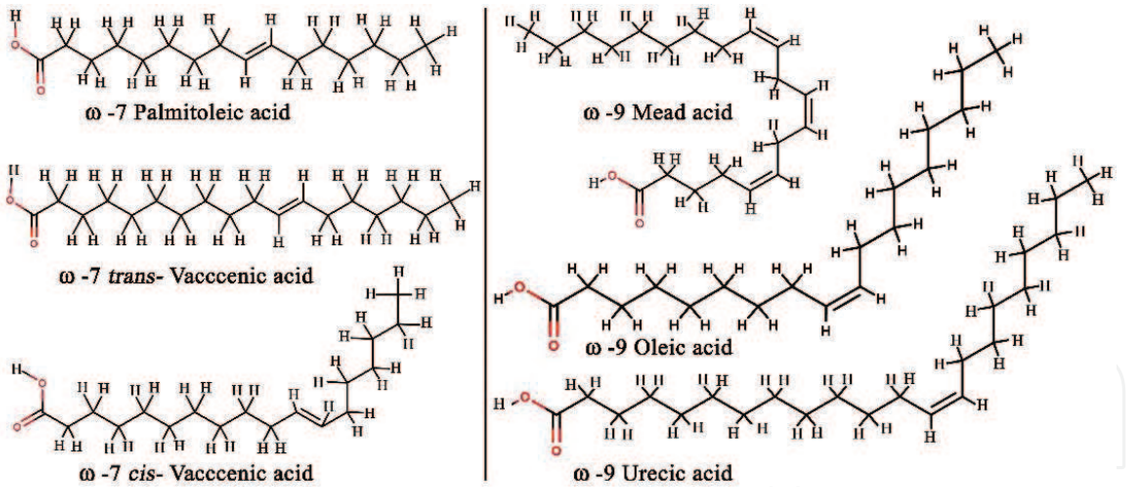

Figure 2.
The structural features of the most common ω-7 and ω-9 unsaturated fatty acids.

1.7 Cis-trans fatty acids

The naturally occurring unsaturated fatty acids have predominantly a *cis* carbon-carbon double bond (-C=C-). The C=C double bond typically lies on C-9 for the C18 unsaturated fatty acids. However, the artificial hydrogenation of C-18 unsaturated fatty acids such as linoleic acid (C18:2, ω-6) may produce *cis-trans* conjugated fatty acid (CLA), like isomers of *cis* Δ-9, *trans* Δ-11; *cis* Δ-9, *trans* Δ-12; and *trans* Δ-10, *cis* Δ-12. Hydrogenation may produce other forms of *trans* fatty acids (TFAs), such as *trans* Δ-8, *trans* Δ-9, and *trans* Δ-10 elaidic acid and *trans* Δ-11 vaccenic acid (**Figure 3**). *Trans* fatty acids (TFAs) are a kind of unsaturated fatty acids and also nonessential fatty acids. The primary TFAs are elaidic acid and vaccenic acid. The vaccenic acid is produced by bacteria in cattle rumen and thus may pass into humans via the milk of cows. The *trans* Δ-9 elaidic acid is the major industrial isomer of TFA [15].

The reports on the effect of CLAs on health and diseases are still scant. Raff et al. [16] reported that a 50:50 mixture of *cis* Δ-9, *trans* Δ-11 CLA and *trans* Δ-10, *cis* Δ-12 CLA caused a nonsignificant increase in SBP (by only 3 mmHg) without any effect on DBP in humans. Laso et al. [17] reported that CLA did not have any effect on blood pressure. Zock and Katan [18] reported that CLAs increase LDL-C and decrease HDL-C, thus indicating that CLA can act as a potential vascular risk factor. American Heart Association, the American Dietetic Association, the Institute of Medicine, US Dietary Guidelines, and the National Cholesterol

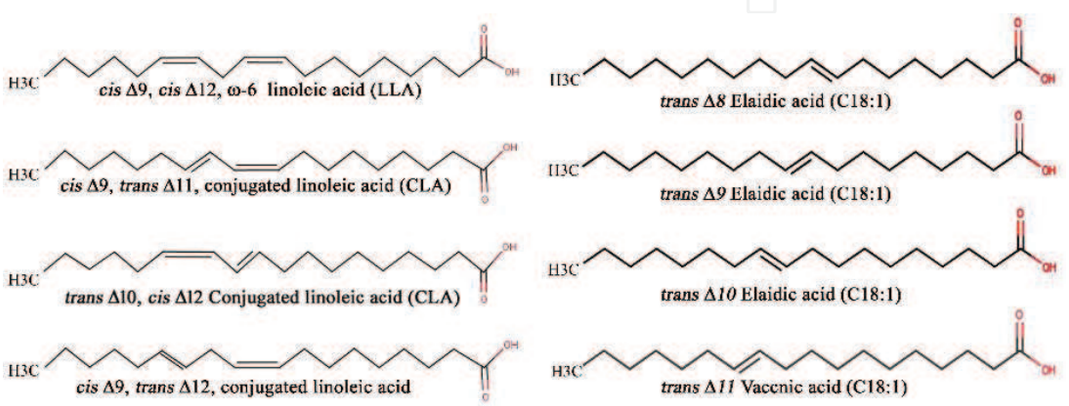

Figure 3.
The structural features of the most common cis-trans unsaturated fatty acids.

Education Program Adult Treatment Panel are claiming to limit *trans* fatty acids in the daily diet [19]. We have previously reported that *cis* Δ-9, *trans* Δ-11-conjugated linoleic acid promotes neuronal differentiation [20] in rats. These reports thus suggest that the effects of CLA remain to be resolved cautiously.

2. Physicochemical properties of fatty acids

Fatty acids are ubiquitous biological molecules. They are esterified to numerous complex lipid molecules, including triglycerides, phospholipids, and cholesterol esters. As being part of these molecules, fatty acids thus may govern some of their physical properties. The aliphatic chains and their lengths confer hydrophobicity to fatty acids. The hydrophobic nature of the fatty acids renders them insoluble in aqueous environments.

At very high pH, where the longer chained fatty acids are totally ionized, they form micelles, which are thermodynamically stable aggregates of molecules in aqueous solution [21]. This property confers the ionized fatty acids to detergent properties. However, to achieve a stable micelle formation, the fatty acids must be present in a solution at a pH greater than 9, which is generally unphysiologic. In fact, the most probable state of fatty acids at physiological temperature and pH is a membrane-like bilayer structure [22] (**Figure 4**, the middle one). The chain length of the fatty acid is interrelated with melting point; the higher the chain length, the lower the melting point. The double bonds in the (poly)unsaturated fatty acids further decrease their melting points [23]. This is very critical to the survival of mammals that live in extremely cold environments such as the polar areas of the earth. The presence of fatty acids in the bilayer membranes provides an excellent anisotropic solution for other membrane constituents. They confer fluidity to the membrane bilayer [24], wherein membrane-bound receptors, enzymes, and other proteins can diffuse laterally along the surface of the bilayer membrane. Phospholipids can also flip-flop between the bilayer leaflets and/or fatty acyl chains can have a vertical motion (translational motion). The word membrane fluidity can thus be referred to as the degree of stiffness or rigidity of the cellular bilayers. As saturated fatty acids are straight-chained, they can pack/stack easily with themselves and/or with the neighbor-cholesterol in the bilayer membrane. The (poly)unsaturated fatty acyl chains, on the other hand, retain bent(s) along the long axis of the chain at the position of double bonds; thus, they cannot align/stack tightly (**Figure 5**).

Consequently, they increase the degree of membrane fluidity. Therefore, the greater the degree of unsaturation of the fatty acids, the higher the fluidity of the membrane. We have previously reported the DHA, which has six double bonds, contributes to a greater extent in membrane fluidity than less-unsaturated

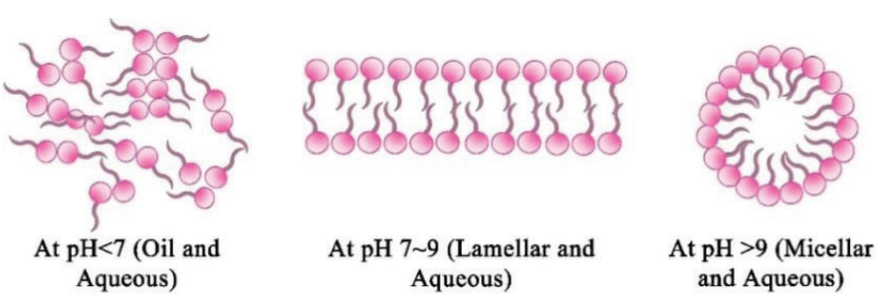

Figure 4.
The arrangements of fatty acids in aqueous environments at T > Tc. T = temperature. Tc = melting point of the fatty acid.

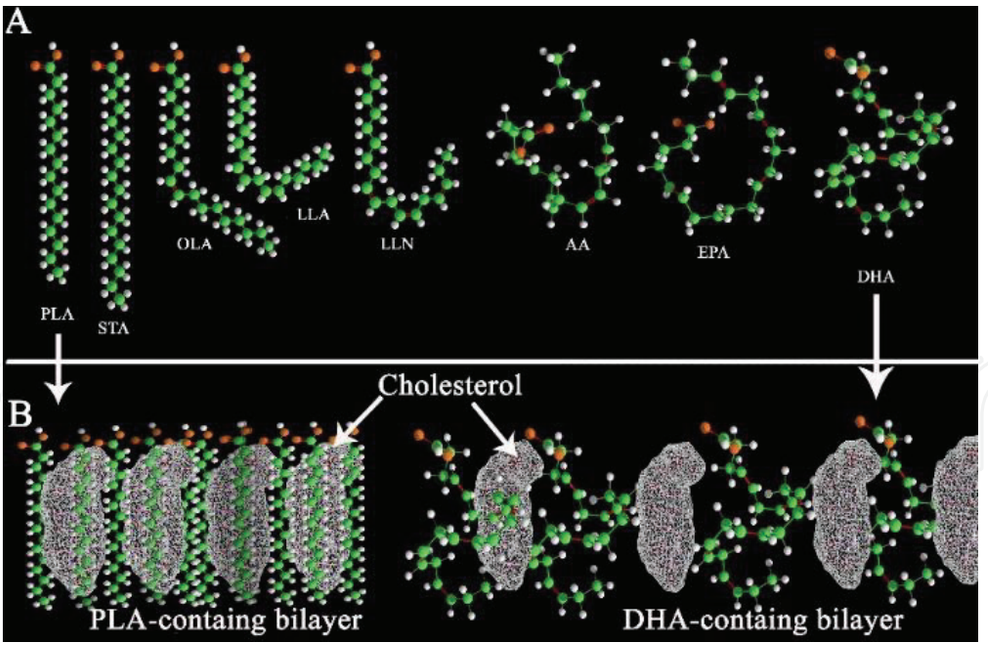

Figure 5.
The 3D structural features of the most common fatty acids (A). PLA = palmitic acid, STA = stearic acid, OLA = oleic acid, LLA = linoleic acid, LLN = α-linolenic acid, AA = arachidonic acid, EPA = eicosapentaenoic acid, DHA = docosahexaenoic acid. Double bonds of the unsaturated fatty acids are denoted by red color (in A) (B). Because of the presence of double bond(s) along the long axis of (poly)unsaturated fatty acids, they occupy more space when they are esterified in the phospholipid bilayer and loosely align with 3D cholesterol, and increase the degree of disorder (membrane fluidity). However, when straight-chained saturated fatty acids like PLA highly align (stacks) with 3D cholesterol, the degree of packing in the bilayer increases (tightens); hence, the membrane bilayer becomes more rigid, that is, less fluid.

fattyvacids, such as EPA and/or saturated fatty acids [25, 26]. As a whole, the physicochemical properties of the fatty acids may affect the functions of these molecules [27], ultimately leading to altered functions of the cells and organisms.

3. Fatty acid oxidation

Fatty acids are usually oxidized by most of the cells of tissues in the body, except the RBCs. The cells of the central nervous system also do not use fatty acids for their energy requirements, using instead carbohydrates or ketone bodies. Heart cell fully depends on energy derived from fatty acid oxidation. Fatty acids constitute the principal source of energy for cells between meals, during hypoglycemia, and/or in diabetes. Beta-oxidation of fatty acids takes place in the mitochondria and, to some extent, in the peroxisomes, particularly the very long chain fatty acids [28]. Unlike in the mitochondria, beta-oxidation of fatty acid in the peroxisomes is not coupled to ATP; the high-potential electrons are rather transferred to O_2, yielding hydrogen peroxide (H_2O_2) and generating heat. The enzyme catalase, found exclusively in peroxisomes, converts H_2O_2 into water and oxygen. H_2O_2 is also used intracellularly to digest unwanted wastes like proteins and/or to defend against intracellular foreign particles including toxins or microorganisms. All fatty acids are not oxidized at the same rates, which implicates that the purposes of cellular accumulation of fatty acids are not the same for all cells. Some fatty acids might have been exploited for energy purposes, some of them might be exploited for the structural purposes, and some of them (or their derivatives) might help the cell for the signal transductions. For example, 30–40% of all esterified fatty acids in the neural plasma membrane phospholipids consist of DHA [29], while EPA constitutes only a tiny percent of the brain total fatty acid. Among the saturated fatty acids, lauric acid (12:0) is oxidized

at the fastest rate and is the most efficient energy substrate[30]. Oleic acid (18:1) is also oxidized at a remarkably faster rate, similar to that of lauric acid. Of the ω-6 essential fatty acids studied, linoleic acid (18:2, ω-6) is oxidized at a faster rate, with arachidonic acid (20:4, ω -6) being oxidized at the slowest rate. DHA and EPA possess different oxidizing properties [31, 32]. DHA is a poor substrate for both mitochondrial and peroxisomal beta-oxidation [33], while EPA can be oxidized and to a much greater extent than DHA [33, 34]. The mechanisms of these properties are not fully elucidated, although intensive investigations are continuously going on. Furthermore, ω-3 fatty acids are incorporated into cell membranes in a highly selective manner where they act as structural components influencing fluidity of the membrane [35]. The ω-3 fatty acids also compromise themselves for enzymatic biotransformations into eicosanoids/docosanoids that act as intracellular signaling molecules and, finally, they get involved in the activity of membrane-bound enzymes, ion channels, and receptors [36]. When EPA is administered to rats, both the EPA and DHA accumulate in different organs, including brain [37], indicating EPA is elongated to DHA. DHA administration also leads to an accumulation of EPA both in the plasma and brains, however, only a tiny percent [37]. As DHA seems difficult to metabolize, we thus speculate that DHA is retroconverted to EPA for further metabolism. Therefore, EPA and DHA imply different metabolic properties in the cells of the brains.

4. Roles of ω-6 and ω-3 PUFAs in physiology

4.1 Platelet physiology

Platelets are derived from megakaryocytes and cause aggregation and play important roles in physiological conditions and pathological conditions as well. Fatty acids are enriched in the plasma membranes of platelets and thus may contribute to the physiology and pathology of platelets. Oral administration of ω-3 PUFAs to rats decreases the degree of platelet aggregation both in rats and humans [38, 39]; hence, it is evident that fatty acids may affect the platelet physiology and atherosclerosis. The mechanisms through which PUFA affects the platelet aggregation is unclear; however, it is assumed that ω-3 PUFA deceases the levels of atherogenic ω-6 PUFA particularly platelet membrane-AA, which acts as a proaggregatory fatty acid. Therefore, ω-3 prevents platelet aggregation by inhibiting PLA2 and interrupting the prostaglandin/thromboxane pathways [40, 41]. In addition, ω-3 PUFAs modulate the platelet membrane fluidity [42], specific lipid domains that hold the receptors for a variety of aggregation factors, such as ADP, thrombin, fibrin, etc. [37], and doing so, they decrease platelet aggregation.

4.2 Effects of fatty acids on hypertension

The effect of fish oil on hypertension came into light when the Norwegian under Nazi invasion had to consume more fish rather than land-based food items during WWII [43]. The Norwegian had low blood pressure, low degree of platelet aggregation, and hypocholesterolemia as well. Afterwards, in studies on the Greenlandic Eskimos, Dyerberg and Bang [44] and Fischer et al. [45] reported that the Eskimos had also a low incidence hypertension and blood cholesterol levels. Then, oil components of marine animals and fish, in particular EPA and DHA, were attributed to lower incidence of cardiovascular risk factors, such as hypertension, hypercholesterolemia, and platelet hyperaggregation. We have previously reported that oral administration of EPA and DHA to rats

(hypercholesterolemic) decreased the hypertension [46] and hypercholesterolemia [47]. The results were consistent with many other published reports [48]. To understand the mechanism(s) of action of these PUFAs, we also pretreated the rat thoracic endothelial cells with these PUFAs and some interesting data emerged from our experiments. For example, the EPA and DHA increased the plasma levels of nucleotide products including ATP, ADP, AMP, and adenosine. The blood vessels of the PUFA-fed rats exhibited less sensitivity to noradrenaline and had caused an increased release of the total purines (ATP + ADP + AMP + Adenosine), concurrently with less contractility [47]. We hypothesized that these nucleotides and their derivatives decreased the noradrenaline sensitivity to purine-receptors of the blood vessels and decreased the blood pressure. The mechanism also might be related to the EPA/DHA-induced increase in the membrane fluidity of the endothelial cells (ECs). These hypotheses led us to preincubate the cultured ECs with EPA and DHA. As expected, the PUFAs increased the membrane fluidity of the ECS [49]. The inhibitory effects of fish oil ω-3 polyunsaturated fatty acids (PUFAs) have also been reported on the expression of endothelial cell adhesion molecules [50]. Hence, the ω-3 PUFAs might have played beneficial roles in reducing hypertension in the animal models as well as in human cases who consumed fish/marine animals' oils in their everyday life.

4.3 Effects of fatty acids on hepatic functions

Saturated and/or unsaturated fatty acids are indispensable for the functions of all tissues in the mammalian body. However, an adequate balance between saturated and (poly)unsaturated and between ω-6 and ω-3 PUFAs is essential to the proper functioning of the cells. Fatty acids after their absorption in the intestinal epithelial cells are first carried to the liver, which acts as a distribution center for the whole body. Therefore, fatty acids can affect the liver functions. Inadequate amounts of essential fatty acids may cause disorders of the liver, such as fatty liver, liver cirrhosis, metabolic syndromes, hyperlipidemia, hypercholesterolemia, and other liver problems [51, 52].

Oral administration of ω-3 DHA decreases the plasma as well as hepatic cholesterol and triacylglycerol levels [53]. The mechanism through which ω-3 PUFAs decrease the plasma cholesterol is not clear; however, it is attributed to the inhibition of hepatic HMG-CoA reductase by the PUFAs, including EPA and DHA. To prove the mechanism, we determined the levels of hepatic mRNA levels of HMG-CoA reductase (yet unpublished) of the DHA-fed rats. DHA decreased the expression of HMG-CoA reductase. Our results were also consistent with numerous other published reports [54–56]. The beneficial effects also emerged at lower levels of LDL-C and TG and high levels of HDL-C. The oral administration of DHA also increased the levels of ω-3 PUFAs and decreased the levels of ω-6 AA both in the plasma and liver tissues. It might be suggested that the oral administration of PUFAs like DHA increases the degrees of oxidative stress and mammalian tissues, including the liver. However, the levels of lipid peroxide (LPO) and reactive oxygen species (ROS) were not increased, thus demonstrating that the feeding of DHA does not pose an oxidative stress to the tissues. We suggest that the oral administration of DHA rather increases the levels of antioxidative enzymes, including glutathione peroxidase and catalase, and antioxidant substrate like GSH [53]. In a similar study, the levels of antioxidative enzymes and GSH increased in the brains of hypercholesterolemic aged rats after oral administration of DHA [57]. However, there are also contradictory results where consumption of PUFA was reported to promote oxidative stress [58]. Furthermore, we isolated and purified the canalicular plasma membranes of the hepatic cells of EPA/DHA-fed rats.

These membranes allow the transport and pump bile components in-and-out of the hepatic cells. The levels of PUFAs increased in the canalicular plasma membranes, concurrent with increases in the activities of membrane-bound enzymes such as Mg^{2-}-ATPase, 5′-nucleotidase. Membrane fluidity also increased in these membranes, thus suggesting that an increased fluidity might have helped in the pumping out the cholesterol via the bile (**Figure 6**). Otherwise, the levels of fecal cholesterol could have not been increased in the feces of the fish-oil-fed rats [53].

4.4 Anti-inflammatory responses

ω-6 PUFA like arachidonic acid (AA, C20:4, ω-6) generates 2-series prostanoids, namely prostaglandins PGE_2, PGI_2, PGD_2, and $PGF_2\alpha$ (largely produced by monocytes and macrophages) and thromboxanes TXA_2 and TXB_2 by COX-1/COX-2 enzymes. Prostaglandin PGI_2/PGE_2 has proinflammatory effects. AA by the action of LOX also produces leukotrienes such as 5-HETE and 5-HPETE, LTE_4, LTB_4, LTC_4, and LTD_4. They are strong proinflammatory agents and have vasoconstriction effects and platelet- and/or neutrophil- and macrophage-activating effects [59–61]. Interestingly, the eicosanoids derived from the action COX and/or LOX on EPA and DHA produces 3-series prostaglandins and thromboxanes and 5-series leukotrienes, and they are less inflammatory and even have anti-inflammatory effects, as compared to the eicosanoids derived from AA. These lipid mediators antagonize the effects of those derived from AA, thus conferring beneficial effects on inflammatory responses [62].

4.5 Effects on skeletal muscles

Skeletal muscle is the largest organ in the human body, comprising approximately 40% of total body weight [63]. This muscle has a plastic-like property and has adapting capability to physical activity. Strenuous muscle exercise increases muscle fatigue and decreases muscle strength, leading to an increase in muscle oxidative stress. It is believed that the response of skeletal muscle to exercise can be modified by the nutritional status of the muscles. There

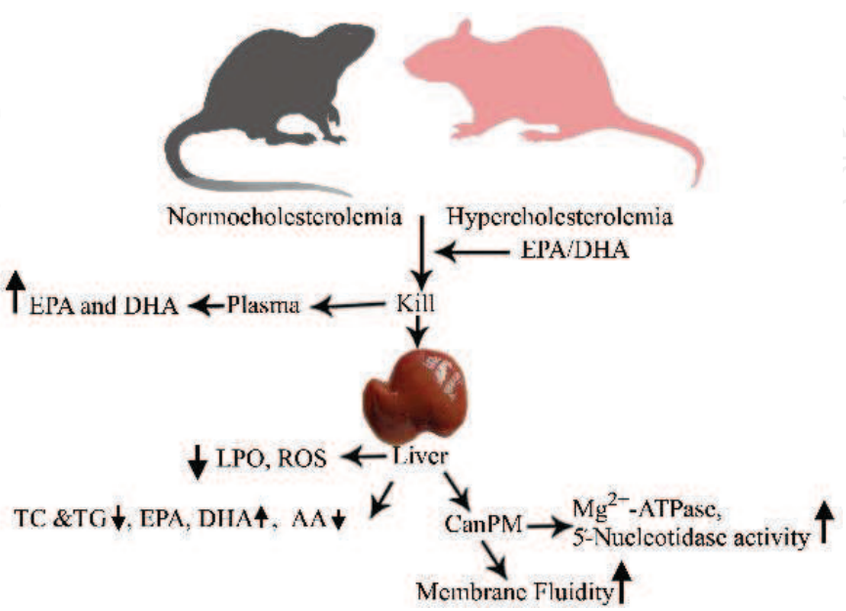

Figure 6.
Effects of oral administration of EPA and DHA on the plasma and hepatic lipid profile (TC = total cholesterol, TG = triacylglycerol), LPO = lipid peroxide, ROS = reactive oxygen species, CanPM = canalicular plasma membrane of hepatic cells).

are numerous reports on the beneficial effects of EPA and DHA on muscle. Therefore, the effects of these PUFAs on muscle strength have been investigated with increasing interest. Hess et al. [64] reported that dietary algae and marine fish increase the levels of EPA and DHA in the equine skeletal muscles. Guen et al. [65] reported that DHA-enriched supplementation improves endurance exercise capacity and skeletal muscle mitochondrial function in murine skeletal muscle. Stebbins et al. [66] reported that DHA + EPA enhances skeletal-muscle blood and vascular conductance in active skeletal muscle (especially type I and IIa fibers) and that the increase in muscle blood is due to an increase in cardiac output secondary to increases in vascular conductance [66]. However, we believe that there are differential effects of PUFAs on the muscle [67]. AA deposition in the fast-twitch muscle of aging rats reduced cell volume with an increase in oxidative stress [68].

5. Effect of ω-3 DHA/EPA on brain cognition

As neurons are the structural and functional units of brain, electrochemical properties of the neurons allow them to transmit signals over long distances and send information to each other. Neurons form the basis of the brain activity and brain cognition and dictate the whole body when and how to work and maintain the behavior of the animals, including humans. Numerous reports have been published stating that the PUFAs have colossal roles in brain growth and development, learning, and memory. At the same time, deficiency of PUFAs such as DHA has been reported to cause neurodegeneration leading to impairments of memory and brain cognition.

Henriksen et al., reported that the level of DHA was low in the preterm infants (born at <33 weeks gestation, body weight < 1.5 Kg). Concurrently, the preterm infants had learning disabilities, reduced IQ, and weak visuospatial relations. However, when these infants were supplemented with DHA, they exhibited normal growth and development in terms of body weight, height, head circumference, visual acuity, and mental development [69]. The study thus suggests that DHA is important before birth. Infants (9-month-old, growth spurt period) fed with DHA-supplemented traditional formula showed higher problem-solving activities, when compared with those fed with traditional formula-only, suggesting thus that DHA also plays an important role during growth spurts and development [70]. Infant's gray matter autopsy (of human/nonhuman primate) study showed that brain DHA levels have also 40% higher in the DHA-supplemented formula-fed infants than those in the formula-fed only infant brains [29, 71]. In addition, DHA declines in aging and age-related neurodegenerative diseases such as Alzheimer's disease [72–74]. All these investigations thus suggest that DHA is important for brain cognitions, such as learning and memory, thought processes, tracing of new information, and comprehension, and that brain DHA deficiency can be recovered by the dietary DHA supplementation. Though cerebral endothelial cells and brain astroglial cells can synthesize DHA and/or α-LLN, EPA from the diet can act as precursors for the DHA; however, the endogenous synthesis or conversion of DHA is extremely low [75] . Thus, dietary DHA is the ultimate source for the DHA in the brain.

We have previously reported that oral administration of DHA for 12 weeks significantly increased the learning-related memory, as evaluated by the 8-armed-radial maze task in DHA-deficient young and old rats [76, 77]. Not only DHA increases the memory of DHA-deficient young and old rats, DHA

also had an extraordinary ability to increase the learning-related memory of Alzheimer's disease model rats [78, 79] (**Figure 7**). EPA also increased the learning-related memory, however, only after their conversion into DHA [80]. The roles of ω-6 AA on the brain cognitions have also recently been investigated; however, the results are controversial. Memory-enhancing effect of AA has been reported previously [81]. In our investigation, the ω-6 AA failed to increase memory of rats (yet unpublished). DHA always exhibited a positive effect on memory. However, the mechanisms by which DHA increases the memory remain to be clarified. Numerous mechanisms of action of DHA on memory have been proposed. DHA-induced increases in synaptic plasma membrane fluidity [26]; antioxidative effects [76–79]; anti-apoptotic effect [78]; increased expressions memory-related proteins, including postsynaptic PSD-95, presynaptic synaptophysin, NMDA-receptor unit NR2A [75], and c-fos [82]; and reductions of brain Aβ-burdens [83] have been attributed to the beneficial effects of DHA in the normal and AD rats, respectively. To examine the mechanism(s) of the reduction of amyloid burden, we tested whether DHA affects the *in vitro* Aβ peptide ($A\beta_{25-35}$, $A\beta_{1-40}$, and $A\beta_{1-42}$ are the most toxic amyloids) fibrillation, a process that assumes to increase the Aβ deposition in the brains. We found that DHA inhibits *in vitro* Aβ fibrillation both at the initial stage of Aβ-seed formation and oligomerization and also causes dissolution of mature Aβ peptide fibers [84–86] (**Figure 8A**). It is thus conceivable that DHA, by decreasing the amyloid fibrillation, decreases the brain Aβ-burden and hence contributes to the amelioration of memory of AD model rats. DHA also caused a clearance of circulating Aβs by increased lipid raft-dependent degradation pathways [87].

We later tested whether DHA affects neurogenesis, which is of great interest in the modulation of memory both in the aging and neurodegenerative Alzheimer's disease. As expected, DHA accelerated both *in vitro* and *in vivo*

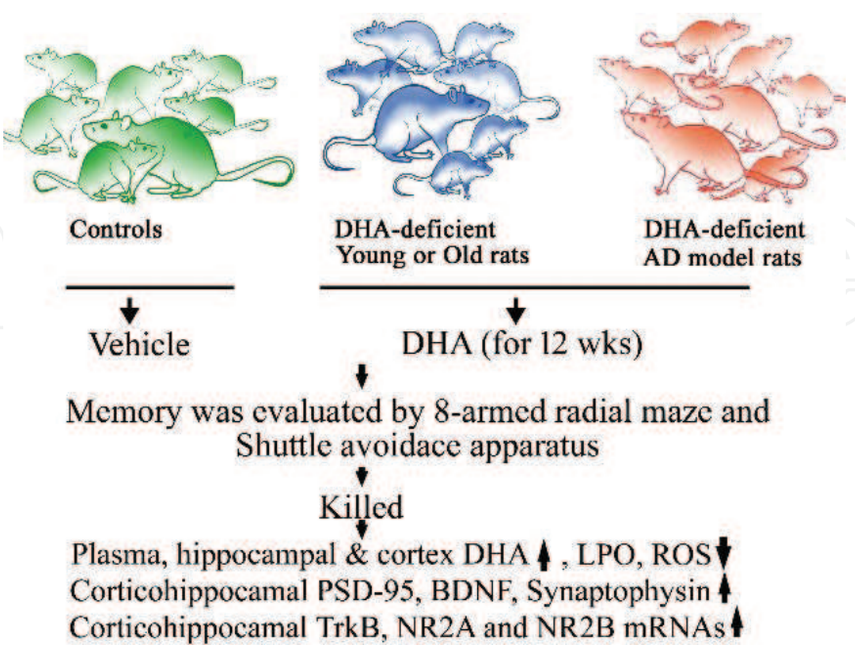

Figure 7.
Effect of oral administration of DHA on the learning-related memory of DHA-deficient young/old and Alzheimer's disease model rats. Protein levels of postsynaptic density protein (PSD-95), brain-derived neurotropic factor (BDNF), and presynaptic synaptophysin were measured. Also, the mRNA levels of BDNF-receptor tyrosine Kinase B (TrkB) and NMDA receptor units NR2A and NR2B were determined by RT-PCR to examine whether they were affected by the oral administration of exogenous DHA. All these parameters were ameliorated by the oral administration of DHA.

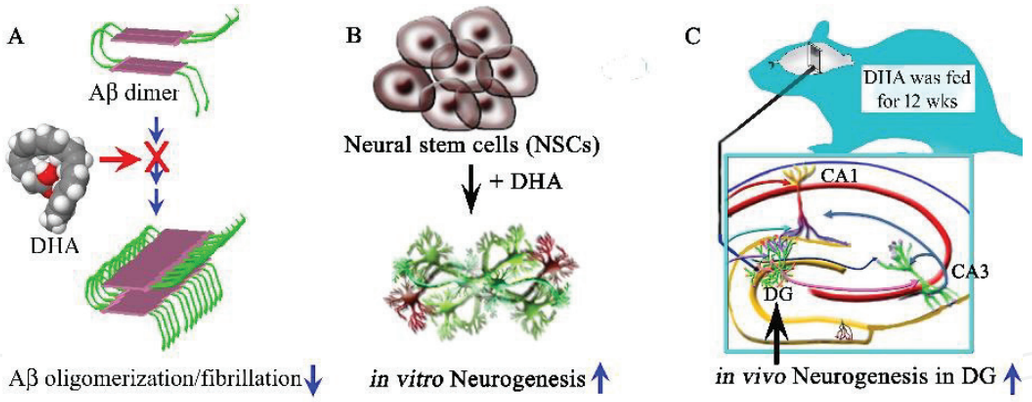

Figure 8.
Effect of incubation of DHA on in vitro amyloid beta (Aβ) peptide fibrillation (A) and in vitro neurogenesis in NSCs culture (B) and, effect of oral administration of in vivo neurogenesis (C). Neurogenesis occurred primarily in the dentate gyrus (DG) region.

neurogenesis [88] (**Figure 8B, C**), which is conducive to inhibition of the impairments of memory in aging and/or AD model rats. DHA stimulated the differentiation of neural stem cells into mature neurons by triggering the activating-type bHLH transcription factors, including neurogenin, Mash1, and NeuroD and inhibiting the repressor-type transcription factor Mes1 [89]. We also reported that DHA-derived docosanoids, such as neuroprotectin D1, help increase the memory of rats [90]. Consistent with our results, Bazan et al. [91] also reported that endogenous signaling by DHA-derived mediators sustains neuronal function and protects synapses and circuits, thus demonstrating that DHA and/or its docosanoid products might act as signaling molecules during memory processing. Finally, DHA is essential for the growth and development of brain and might play crucial roles in the formation of learning-related brain cognition.

6. Conclusion

For the last several decades, fatty acid nutrition, in terms of quality, has been dramatically changed [92]. Consumption of saturated fatty acids, ω-6 PUFAs, and *trans* fatty acid intake has been increased [93]. Optimal dietary ω-6:ω-3 ratio should be around 1–4:1; however, this ratio has now increased to 10:1 to 20:1 in the Western diet [92]. Concurrently, the incidence of diseases involving inflammatory diseases, cardiovascular disease, obesity, rheumatoid arthritis, cancer, neurodegenerative, and psychiatric illnesses, such as AD and depression, are increasing with an ever-increasing rate [94]. The results of our investigations and those of the others, finally, suggest that DHA is accumulated in the synaptic plasma membranes, represses oxidative stress by increasing the antioxidative defense, decreases cholesterol in the detergent-insoluble membrane fraction (DIMF) of the brain tissues, increases synaptic plasma membrane fluidity, inhibits amyloid fibrillation and decreases amyloid toxicity and burden in the brain tissues, improves the neuronal morphology, increases memory-related protein substrates, and hence ameliorates the memory-related brain cognition (**Figure 9**). In conclusion, a balanced intake of ω-3 and ω-6 PUFAs is a must, as well as an increased intake of DHA, which might act as a signaling molecule to protect the brains from preterm-, postnatal-, and other age-related neurological diseases, such as Alzheimer's disease.

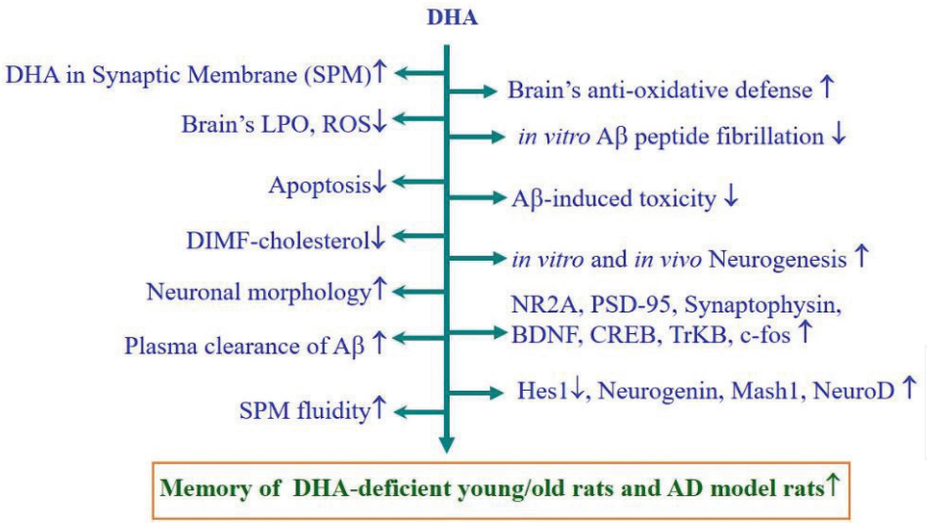

Figure 9.
Outlines of the effect of DHA on learning-related memory of rats. SPM = synaptic plasma membrane. DIMF = detergent-insoluble membrane fraction. All other abbreviations are same as for other figures.

Author details

Michio Hashimoto[1*] and Shahdat Hossain[2]

1 Department of Environmental Physiology, Shimane University Faculty of Medicine, Japan

2 Department of Biochemistry and Molecular Biology, Jahangirnagar University, Savar, Dhaka, Bangladesh

*Address all correspondence to: michio1@med.shimane-u.ac.jp

References

[1] Chevreul ME. Sur plusieurs corps gras, et particulièrement sur leurs combinaisons avec les alcalis. Annales de Chimie. 1823;**88**:225-261

[2] Chevreul ME. Recherches sur les corps gras d'origine animale. Paris: Levrault; 1823

[3] Leray C. Chronological history of lipid center. Cyberlipid Center. Last updated on 16 July 2018. [Internet]. Available from: http://www.cyberlipid.org/cyberlip/home0001.htm [Accessed: 2018-05-25]

[4] Bruno G. Essential and non-essential fatty acids [Internet]. 2005. Available from: https://www.huhs.edu/literature/Essential%20Fatty%20Acids.pdf [Accessed: 2018-05-25]

[5] Le HD, Meisel JA, de Meijer VE, Gura KM, Puder M. The essentiality of arachidonic acid and docosahexaenoic acid. Prostaglandins, Leukotrienes, and Essential Fatty Acids. 2009;**81**: 165-170

[6] Hadley K, Ryan AS, Forsyth S, Gautier S, Salem N. The essentiality of arachidonic acid in infant development. Nutrients. 2016;**8**:216

[7] Burke PA, Ling PR, Forse RA, Bistrian BR. Conditionally essential fatty acid deficiencies in end-stage liver disease. Nutrition. 1999;**15**:302-304

[8] Bates EJ, Ferrante A, Harvey DP, Nandoskar M, PoulosA. Docosahexanoic acid (22:6, n-3) but not eicosapentaenoic acid (20:5, n-3) can induce neutrophil-mediated injury of cultured endothelial cells: Involvement of neutrophil elastase. Journal of Leukocyte Biology. 1993;**54**:590-598

[9] Lokesh BR, German B, Kinsella JE. Differential effects of docosahexaenoic acid and eicosapentaenoic acid on suppression of lipoxygenase pathway in peritoneal macrophages. Biochimica et Biophysica Acta. 1988;**958**:99-107

[10] Shiina T, Terano T, Saito J, Tamura Y, Yoshida S. Eicosapentaenoic acid and docosahexaenoic acid suppress the proliferation of vascular smooth muscle cells. Atherosclerosis. 1993;**104**:95-103

[11] Hashimoto M, Hossain MS, Yamazaki H, Yazawa K, Masumura S. Effects of eicosapentaenoic acid and docosahexaenoic acid on plasma membrane fluidity of aortic endothelial cells. Lipids. 1999;**34**:1297-1304

[12] Hamano H, Nabekura J, Nishikawa M, Ogawa T. Docosahexaenoic acid reduces GABA response I substantia nigra neuron of rat. Journal of Neurophysiology. 1996;**75**:1264-1270

[13] Sanders TAB. DHA status of vegetarians. Prostaglandins, Leukotrienes, and Essential Fatty Acids. 2009;**81**:137-141

[14] Geppert J, Kraft V, Demmelmair H, Koletzko B. Microalgal docosahexaenoic acid decreases plasma triacylglycerol in normolipidaemic vegetarians: a randomized trial. The British Journal of Nutrition. 2006;**95**:779-786

[15] Dhaka V, Gulia N, Ahlawat KS, Khatkar BS. Trans fats-sources, health risks and alternative approach—A review. Journal of Food Science and Technology. 2011;**48**(5):534-541

[16] Raff M, Tholstrup T, Sejrsen K, Straarup EM, Wiinberg N. Diets rich in conjugated linoleic acid and vaccenic acid have no effect on blood pressure and isobaric arterial elasticity in healthy young men. The Journal of Nutrition. 2006;**136**(4):992-997

[17] Laso N, Brugué E, Vidal J, Ros E, Arnaiz JA, et al. Effects of milk supplementation with conjugated linoleic acid (isomers cis-9, trans-11 and trans-10, cis-12) on body composition and metabolic syndrome components. The British Journal of Nutrition. 2007;**98**(4):860-867

[18] Zock PL, Katan MB. Hydrogenation alternatives: Effects of *trans* fatty acids and stearic acid versus linoleic acid on serum lipids and lipoproteins in humans. Journal of Lipid Research. 1992;**33**(3):399-410

[19] Remig V, Franklin B, Margolis S, Kostas G, Nece T, Street JC. Trans fats in America: A review of their use, consumption, health implications, and regulation. Journal of the American Dietetic Association. Apr 2010;**110**(4):585-592

[20] Okui T, Hashimoto M, Katakura M, Shido O. Cis-9, trans-11-conjugated linoleic acid promotes neuronal differentiation through regulation of Hes6 mRNA and cell cycle in cultured neural stem cells. Prostaglandins, Leukotrienes, and Essential Fatty Acids. 2011;**85**(3-4):163-169

[21] Small DM. The physical chemistry of lipids from alkanes to phospholipids. In: Hanahan D, editor. Handbook of Lipid Research. Vol. 4. New York: Plénum Press; 1986. pp. 1-672

[22] Bracco U, Deckelbaum RJ, editors. Polyunsaturated Fatty Acids in Human Nutrition. Vol. 28. Nestle Nutrition Workshop Series. New York: Nestec Ltd., Vevey/Raven Press. Ltd.; 1992

[23] Berg JM, Tymoczko JL, Stryer L. Biochemistry. 5th ed. New York: W.H. Freeman; 2002

[24] Stillwell W, Wassall SR Docosahexaenoic acid: Membrane properties of a unique fatty acid. Chemistry and Physics of Lipids. 2003;**126**(1):1-27

[25] Leekumjorn S, Cho HJ, Wu Y, Wright NT, Sum AK, Chan C. The role of tatty acid unsaturation in minimizing biophysical changes on the structure and local fffects of bilayer membranes. Biochimica et Biophysica Acta. 2009;**1788**(7):1508-1516

[26] Hashimoto M, Hossain S, Shimada T, Shido O. Docosahexaenoic acid-induced protective effect against impaired learning in amyloid beta-infused rats is associated with increased synaptosomal membrane fluidity. Clinical and Experimental Pharmacology & Physiology. 2006;**33**(10):934-939

[27] Gennis RB. Biomembranes: Molecular Structure and Function. Verlag New York: Springer; 1989. ISBN: 038796760

[28] Lazarow PB. Rat liver peroxisomes catalyze the beta oxidation of fatty acids. The Journal of Biological Chemistry. 1978;**253**:1522-1528

[29] Lauritzen L, Hansen HS, Jørgensen MH, Michaelsen KF. The essentiality of long chain n-3 fatty acids in relation to development and function of the brain and retina. Progress in Lipid Research. 2001;**40**:1-94

[30] Leyton J, Drury PJ, Crawford MA. Differential oxidation of saturated and unsaturated fatty acids in vivo in the rat. The British Journal of Nutrition. 1987;**57**(3):383-3893

[31] Willumsen N, Hexeberg S, Skorve J, Lundquist M, Berge RK. Docosahexaenoic acid shows no triglyceride-lowering effects but increases the peroxisomal fatty acid oxidation in liver of rats. Journal of Lipid Research. 1993;**34**:13-22

[32] Madsen L, Frøyland L, Dyrøy E, Helland K, Berge RK. Docosahexaenoic

and eicosapentaenoic acids are differently metabolized in rat liver during mitochondria and peroxisome proliferation. Journal of Lipid Research. 1998;**39**:583-593

[33] Madsen L, Rustan AC, Vaagenes H, Berge K, Dyroy E, Berge RK. Eicosapentaenoic and docosahexaenoic acid affect mitochondrial and peroxisomal fatty acid oxidation in relation to substrate preference. Lipids. 1999;**34**:951-963

[34] Chen CT, Liu Z, Ouellet M, Calon F, Bazinet RP. Rapid beta-oxidation of eicosapentaenoic acid in mouse brain: An in situ study. Prostaglandins, Leukotrienes, and Essential Fatty Acids. 2009;**80**:157-163

[35] Yang X, Sheng W, Sun GY, James C-M, Lee JCM. Effects of fatty acid unsaturation numbers on membrane fluidity and α-secretase-dependent amyloid precursor protein processing. Neurochemistry International. Feb 2011;**58**(3):321-329

[36] Drevon C. Marine oils and their effects. Nutrition Reviews. 1992;**50**:38-45

[37] MacIntyre DE, Hoover RL, Smith M, Steer M, Lynch C, Karnovsky MJ, Salzman EW. Inhibition of platelet function by cis-unsaturated fatty acids. Blood. 1984;**63**(4):848-857

[38] Phang M, Lincz LF, Garg ML. Eicosapentaenoic and docosahexaenoic acid supplementations reduce platelet aggregation and hemostatic markers differentially in men and women. The Journal of Nutrition. 2013;**143**(4):457-463

[39] Nordøy A, Davenas E, Ciavatti M, Renaud S. Effect of dietary (n-3) fatty acids on platelet function and lipid metabolism in rats. Biochimica et Biophysica Acta. 1985; **835**(3):491-500

[40] Vallee E, Gougat J, Ageron M. Inhibition of platelet phospholipase-A2 as a mechanism for the anti-aggregating effect of linoleic acid. Agents and Actions. 1980;**10**(1 Pt 2):57-62

[41] Silver MJ, Smith JB, Ingerman C, Kocsis JJ. Arachidonic acid-induced human platelet aggregation and prostaglandin formation. Prostaglandins. 1973;**4**(6):863-875

[42] Hashimoto M, Hossain S, Shido O. Docosahexaenoic acid but not eicosapentaenoic acid withstands dietary cholesterol-induced decreases in platelet membrane fluidity. Molecular and Cellular Biochemistry. 2006;**293**(1-2):1-8

[43] Bønaa KH. Myocardial infarction mortality. Tidsskrift for den Norske Lægeforening. 2011; **131**(5):440-441

[44] Dyerberg J, Bang HO. Haemostatic function and platelet polyunsaturated fatty acids in Eskimos. Lancet. 1979;**2**(8140):433-435

[45] Fischer S, Weber PC, Dyerberg J. The prostacyclin/thromboxane balance is favourably shifted in Greenland Eskimos. Prostaglandins. 1986;**32**(2):2352-2241

[46] Hashimoto M, Shinozuka K, Shahdat HM, Kwon YM, Tanabe Y, et al. Antihypertensive effect of all-cis-5, 8, 11, 14, 17-icosapentaenoate of aged rats is associated with an increase in the release of ATP from the caudal artery. Journal of Vascular Research. 1998;**35**(1):55-62

[47] Hashimoto M, Shinozuka K, Gamoh S, Tanabe Y, Hossain MS, et al. The hypotensive effect of docosahexaenoic acid is associated with the enhanced release of ATP from the caudal artery of aged rats. Journal of Nutrition. 1999;**129**(1):70-76

[48] Campbell F, Dickinson HO, Critchley JA, Ford GA, Bradburn M. A systematic review of fish-oil supplements for the prevention and treatment of hypertension. European Journal of Preventive Cardiology. 2013;**20**(1):107-120

[49] Hashimoto M, Hossain S, Masumura S. Effect of aging on plasma membrane fluidity of rat aortic endothelial cells. Experimental Gerontology. 1999;**34**(5):687-698

[50] Collie-Duguid ES, Wahle KW. Inhibitory effect of fish oil n-3 polyunsaturated fatty acids on the expression of endothelial cell adhesion molecules. Biochemical and Biophysical Research Communications. 1996;**220**(3):969-974

[51] Simopoulos AP. Evolutionary aspects of diet, the omega-6/omega-3 ratio and genetic variation: Nutritional implications for chronic diseases. Biomedicine & Pharmacotherapy. 2006;**60**:502e7

[52] Yashodhara BM, Umakanth S, Pappachan JM, Bhat SK, Kamath R, Choo BH. Omega-3 fatty acids: A comprehensive review of their role in health and disease. Postgraduate Medical Journal. 2009;**85**:84-90

[53] Hashimoto M, Hossain MS, Shimada T, Yamasaki H, Fujii Y, Shido O. Effects of docosahexaenoic acid on annular lipid fluidity of the rat bile canalicular plasma membrane. Journal of Lipid Research. 2001;**42**(7):1160-1168

[54] Frøyland L, Vaagenes H, Asiedu DK, Garras A, Lie O, Berge RK. Chronic administration of eicosapentaenoic acid and docosahexaenoic acid as ethyl esters reduced plasma cholesterol and changed the fatty acid composition in rat blood and organs. Lipids. 1996;**31**(2):169-178

[55] Choi YS, Goto S, Ikeda I, Sugano M. Effects of dietary n-3 polyunsaturated fatty acids on cholesterol synthesis and degradation in rats of different ages. Lipids. 1989;**24**:45-50

[56] Murthy S, Albright E, Mathur SN, Field FJ. Modification of CaCo-2 cell membrane fatty acid composition by eicosapentaenoic acid and palmitic acid: Effect on cholesterol metabolism. Journal of Lipid Research. 1988;**29**:773-780

[57] Hossain MS, Hashimoto M, Gamoh S, Masumura S. Antioxidative effects of docosahexaenoic acid in the cerebrum versus cerebellum and brainstem of aged hypercholesterolemic rats. Journal of Neurochemistry. 1999;**72**(3):1133-1138

[58] Shefer-Weinberg D, Sasson S, Schwartz B, Argov-Argaman N, Tirosh O. Deleterious effect of n-3 polyunsaturated fatty acids in non-alcoholic steatohepatitis in the fat-1 mouse model. Clinical Nutrition Experimental. 2017;**12**:37-49

[59] Harizi H, Corcuff JB, Gualde N. Arachidonic-acid-derived eicosanoids: Roles in biology and immunopathology. Trends in Molecular Medicine. 2008;**14**(10):461-469

[60] Levin G, Duffin KL, Obukowicz MG, et al. Differential metabolism of dihomo-γ-linolenic acid and arachidonic acid by cyclo-oxygenase-1 and cyclo-oxygenase-2: Implications for cellular synthesis of prostaglandin E-1 and prostaglandin E-2. The Biochemical Journal. 2002;**365**(2):489-496

[61] Bagga D, Wang L, Farias-Eisner R, Glaspy JA, Reddy ST. Differential effects of prostaglandin derived from ω-6 and ω-3 polyunsaturated fatty acids on COX-2 expression and IL-6 secretion. Proceedings of the National Academy

of Sciences of the United States of America. 2003;**100**(4):1751-1756

[62] Robinson JG, Stone NJ. Antiatherosclerotic and antithrombotic effects of omega-3 fatty acids. The American Journal of Cardiology. 2006;**98**(4):39-49

[63] Lowell BB, Shulman GI. Mitochondrial dysfunction and type 2 diabetes. Science. 2005;**307**:384-387

[64] Hess TM, Rexford JK, Hansen DK, Harris M, Schauermann N, Ross T, Engle TE, Allen KG, Mulligan CM. Effects of two different dietary sources of long chain omega-3, highly unsaturated fatty acids on incorporation into the plasma, red blood cell, and skeletal muscle in horses. Journal of Animal Science. 2012;**90**(9):3023-3031

[65] Le Guen M, Chaté V, Hininger-Favier I, Laillet B, Morio B,et al. A 9-wk docosahexaenoic acid-enriched supplementation improves endurance exercise capacity and skeletal muscle mitochondrial function in adult rats. American Journal of Physiology. Endocrinology and Metabolism. 2016;**310**(3):E213-E224

[66] Stebbins CL, Hammel LE, Marshal BJ, Spangenberg EE, Musch TI. Effects of dietary omega-3 polyunsaturated fatty acids on the skeletal-muscle blood-flow response to exercise in rats. International Journal of Sport Nutrition and Exercise Metabolism. 2010;**20**(6):475-486

[67] Hashimoto M, Inoue T, Katakura M, Hossain S, Mamun AA, et al. Differential effects of docoosahexaenoic and arachidonic acid on fatty acid composition and myosin heavy chain-related genes of slow- and fast-twitch skeletal muscle tissues. Molecular and Cellular Biochemistry. 2016;**415**:169-181

[68] Inoue T, Hashimoto M, Katakura M, Tanabe Y, Al Mamun A, et al. Effects of chronic administration of arachidonic acid on lipid profiles and morphology in the skeletal muscles of aged rats. Prostaglandins, Leukotrienes, and Essential Fatty Acids. 2014;**91**(4):119-127

[69] Henriksen C, Haugholt K, Lindgren M, Aurvåg AK, Rønnestad A, et al. Improved cognitive development among preterm infants attributable to early supplementation of human milk with docosahexaenoic acid and arachidonic acid. Pediatrics. 2008;**121**:1137

[70] Drover J, Hoffman DR, Castañeda YS, Morale SE, Birch EE. three randomized controlled trials of early long-chain polyunsaturated fatty acid supplementation on means-end problem solving in nine-month olds. Child Development. 2009;**80**(5): 1376-1384

[71] Wang B, McVeagh P, Petocz P, Brand-Miller J. Brain ganglioside and glycoprotein sialic acid in breastfed compared with formula-fed infants. The American Journal of Clinical Nutrition. 2003;**78**(5):1024-1029

[72] Soderberg M, Edlund C, Kristensson K, Dallner G. Fatty acid composition of brain phospholipids in aging and in Alzheimer's disease. Lipids. 1991;**26**:421-425

[73] Lukiw WJ, Cui JG, Marcheselli VL, Bodker M, Botkjaer A, et al. A role for docosahexaenoic acid- derived neuroprotectin D1 in neural cell survival and Alzheimer disease. The Journal of Clinical Investigation. 2005;**115**:2774-2783

[74] Astarita G, Jung KM, Berchtold NC, Nguyen VQ, Gillen DL,et al. Deficient liver biosynthesis of docosahexaenoic acid correlates with cognitive

impairment in Alzheimer's disease. PLoS One. 2010;**5**:1-8

[75] Hashimoto M, Hossain S, Al Mamun A, Matsuzaki K, Arai H. Docosahexaenoic acid: one molecule diverse functions. Critical Reviews in Biotechnology. 2017;**37**(5):579-597

[76] Gamoh S, Hashimoto M, Hossain S, Masumura S. Chronic administration of docosahexaenoic acid improves the performance of radial arm maze task in aged rats. Clinical and Experimental Pharmacology & Physiology. 2001;**28**(4):266-270

[77] Gamoh S, Hashimoto M, Sugioka K, Shahdat Hossain M, Hata N, Misawa Y, Masumura S. Chronic administration of docosahexaenoic acid improves reference memory-related learning ability in young rats. Neuroscience. 1999;**93**(1):237-241

[78] Hashimoto M, Hossain S, Shimada T, et al. Docosahexaenoic acid provides protection from impairment of learning ability in Alzheimer's disease model rats. Journal of Neurochemistry. 2002;**81**:1084-1091

[79] Hashimoto M, Tanabe Y, Fujii Y, Kikuta T, Shibata H, Shido O. Chronic administration of docosahexaenoic acid ameliorates the impairment of spatial cognition learning ability in amyloid beta-infused rats. The Journal of Nutrition. Mar 2005;**135**(3):549-555

[80] Hashimoto M, Hossain S, Tanabe Y, Kawashima A, Harada T, et al. The protective effect of dietary eicosapentaenoic acid against impairment of spatial cognition learning ability in rats infused with amyloid beta (1- 40). The Journal of Nutritional Biochemistry. 2009;**20**(12):965-973

[81] Li C, Wang Q, Li L, Liu Y, Diao H. Arachidonic acid attenuates learning and memory dysfunction induced by repeated isoflurane anesthesia in rats. International Journal of Clinical and Experimental Medicine. 2015;**8**(8):12365-12373

[82] Tanabe Y, Hashimoto M, Sugioka K, Maruyama M, Fujii Y, et al. Improvement of spatial cognition with dietary docosahexaenoic acid is associated with an increase in Fos expression in rat CA1 hippocampus. Clinical and Experimental Pharmacology & Physiology. 2004;**31**(10):700-703

[83] Hashimoto M, Hossain S, Agdul H, Shido O. Docosahexaenoic acid-induced amelioration on impairment of memory learning in amyloid beta-infused rats relates to the decreases of amyloid beta and cholesterol levels in detergent-insoluble membrane fractions. Biochimica et Biophysica Acta. Dec 30, 2005;**1738**(1-3):91-98

[84] Hashimoto M, Shahdat HM, Yamashita S, Katakura M, Tanabe Y, et al. Docosahexaenoic acid disrupts in vitro amyloid beta (1-40) fibrillation and concomitantly inhibits amyloid levels in cerebral cortex of Alzheimer's disease model rats. Journal of Neurochemistry. 2008;**107**(6):1634-1646

[85] Hossain S, Hashimoto M, Katakura M, Miwa K, Shimada T, Shido O. Mechanism of docosahexaenoic acid-induced inhibition of in vitro Abeta1-42 fibrillation and Abeta1-42-induced toxicity in SH-S5Y5 cells. Journal of Neurochemistry. 2009;**111**(2): 568-579

[86] Hashimoto M, Shahdat HM, Katakura M, Tanabe Y, Gamoh S, et al. Effects of docosahexaenoic acid on in vitro amyloid beta peptide 25-35 fibrillation. Biochimica et Biophysica Acta. 2009;**1791**(4):289-296

[87] Hashimoto M, Hossain S, Katakura M, Mamun AA, Shido O. The binding of Aβ 1-42 to lipid rafts of RBC is enhanced by dietary docosahexaenoic acid in rats: Implicates to Alzheimer's disease. Biochimica et Biophysica Acta. 1848;**2015**:1402-1409

[88] Kawakita E, Hashimoto M, Shido O. Docosahexaenoic acid promotes neurogenesis in vitro and in vivo. Neuroscience. 2006;**139**(3):991-997

[89] Katakura M, Hashimoto M, Shahdat HM, Gamoh S, Okui T, Matsuzaki K, Shido O. Docosahexaenoic acid promotes neuronal differentiation by regulating basic helix-loop-helix transcription factors and cell cycle in neural stem cells. Neuroscience. 2009;**160**(3):651-660

[90] Hashimoto M, Katakura M, Tanabe Y, Al Mamun A, Inoue T, et al. N-3 fatty acids effectively improve the reference memory-related learning ability associated with increased brain docosahexaenoic acid-derived docosanoids in aged rats. Biochimica et Biophysica Acta. 2015;**1851**(2):203-209

[91] Bazan NG, Musto AE, Knott EJ. Endogenous signaling by omega-3 docosahexaenoic acid-derived mediators sustains homeostatic synaptic and circuitry integrity. Molecular Neurobiology. 2011;**44**(2):216-222

[92] Olivier MC, Vanessa L, Isabelle A. Why and how meet n-3 PUFA dietary recommendations? Gastroenterology Research and Practice. 2011;**2011**:1-11. Article ID: 364040

[93] Simopoulos AP. Evolutionary aspects of diet: The omega-6/omega-3 ratio and the brain. Molecular Neurobiology. 2011;**44**(3):203-215

[94] Corsinovi L, Biasi F, Poli G, Leonarduzzi G, Isaia G. Dietary lipids and their oxidized products in Alzheimer's disease. Molecular Nutrition & Food Research. 2011;**55**(Suppl 2):S161-S172